STUDIES ON THE RELATION BETWEEN AMITOSIS AND MITOSIS.

III. Maturation, Fertilizaton, and Cleavage in Moniezia.

C. M. CHILD.

Since the present paper is concerned primarily with the rôle played by amitosis and mitosis, respectively in the later history of the ovum and the early development of *Moniezia*, the stages of egg-maturation and fertilization are in themselves of secondary importance. It has seemed advisable, however, to include an account of these stages, although it has not been possible to attain certainty on a number of points, *e. g.*, the polarity of the egg before maturation, the direction of division of the chromosomes in maturation, the origin of the cleavage centrosomes, etc. Failure to reach definite conclusions upon these points is due, at least in large measure, as will appear, to the character of the material rather than to insufficient observation. Much time and labor has been expended in the attempt to obtain positive data upon these points but thus far without success.

The cleavage of the egg is considered from a cytological rather than a morphological standpoint, and much that is of importance embryologically is not discussed, as aside from the present purpose. All the figures of maturation, fertilization, and almost all of those of cleavage-stages are from *Moniezia expansa* but no essential differences have been discovered in the two species. The magnification is the same as that used in preceding papers of the series.[1]

I. Maturation of the Egg.

In point of time the entrance of the spermatozoön precedes the process of maturation and the growth of the male pronucleus occurs during the formation of the polar bodies. For the sake

[1] Child, C. M., "Studies on the Relation between Amitosis and Mitosis. I., Development of the Ovaries and Oögenesis in Moniezia." Biol. Bull., Vol. XII., No. 2, 1907. II., Development of the Testes and Spermatogenesis in Moniezia. Biol Bull., Vol. XII., Nos. 3 and 4, 1907.

of clearness, however, the two processes are described separately, the figures being sufficient to show the conditions as regards both maturation and fertilization at different stages.

When the egg leaves the ovary on its way to the uterus it is flattened or irregular in shape, without any visible polar differentiation so far as could be discovered, and contains a large nucleus with very large nucleolus. Within the nucleus all traces of the spireme which appeared at the beginning of the growth period[1] have disappeared and except for the nucleolus the nuclear contents, like those of many other egg-nuclei at this stage do not take nuclear stains.

Fig. 1 (Pl. II.) shows an egg at this stage but with the spermatozoön entering. In this egg two bodies shown below the nucleus are probably the centrosomes of the first maturation spindle, though it was impossible to be certain on this point.

With the entrance of the spermatozoön a vitelline membrane is formed (Fig. 2 et seq., Pl. II.). Chromosomes soon begin to form in the nucleus (Figs. 2 and 3, Pl. II.).

In Fig. 4 (Pl. II.) an early stage of the first maturation spindle is shown. Here the outline of the nucleus is still visible and the spindle appears to be wholly intranuclear. The very large centrosomes at the poles show a distinctly differentiated outer boundary which appears almost like a membrane. One of them in this figure shows two deeply staining granules, the other, none. The appearance and division of these central granules, or centrioles, is seemingly rather irregular as the following figures indicate. In no case has any trace of asters been observed at any stage of maturation, but with the formation of the spindle, the yolk spherules arrange themselves about the equatorial region. This arrangement of yolk spherules indicates that conditions about the poles of the spindles are similar to those in species where distinct asters appear. It is probable that the absence of asters is not due to any fundamental difference in the character of the processes in this case as compared with cases where asters are visible, but rather to the nature of the protoplasm or perhaps to the energy of the processes involved. The achromatic spindle-structures themselves are exceedingly delicate and often only

[2] Child, C. M., Biol. Bull, XII., 2, 1907.

very faintly visible. It is possible that some method of fixation which I have not employed may serve to render these phenomena more clearly visible. In any case, however, it seems improbable that the presence or absence of visible asters can be regarded as of essential importance.

It has not been possible to determine with certainty the number of chromosomes, though it is not far from eight, the most frequent number in the spermatocytes.[1] The arrangement of the chromosomes upon the spindle is very irregular and anything approaching a typical equatorial plate is rarely seen. The chromosomes exhibit the form characteristic of heterotypic divisions, but no conclusions were possible regarding the direction of division. Figs. 5 and 6 (Pl. II.) show other examples of the first maturation spindle. In Fig. 6 the extremely irregular passage of the chromatic material to the two poles is shown. The chromosomes often appear to be more or less broken up into chains of granules, which seem in some cases to become wholly separated from each other. It is impossible, of course, to determine by observation whether the chromosomes maintain their individuality during this process, but cases of this kind certainly do not appear to strengthen the hypothesis of individuality.

In some cases a nucleus with distinct membrane is formed after the first maturation division (Fig. 7, Pl. II.). In other cases, and apparently more frequently, the second polar spindle appears without an intervening resting stage (Fig. 8, Pl. II.).

The first polar body is of large size (Figs. 7 and 8, Pl. II.) and the centrosome is frequently visible beside the nucleus in it (Fig. 8, Pl. II., Figs. 9 and 10, Pl. III.). This centrosome stains more deeply than in earlier stages and still later apparently undergoes condensation to such an extent that it stains as deeply with iron-hæmatoxylin as a mass of chromatin or a yolk granule.

Fig. 8 (Pl. II.) represents an early stage of the second maturation spindle and Figs. 9 and 10 (Pl. III.) later stages. Fig. 10 is one of the very rare cases in which anything like a typical anaphase has been observed. The change in position of the spindle during its development is apparent by comparison of Fig. 8 (Pl. II.) with Fig. 9 (Pl. III.).

[1] Child, C. M., Biol. Bull., XII., 4, 1907.

The second polar body is, as is usual, smaller than the first (Fig. 11, Pl. III.) both as regards nucleus and cytoplasm. Division has not been observed in either polar body, though in one case what appeared to be an abnormal or abortive mitosis was seen in the first polar body.

Fig. 11 (Pl. III.) shows the reconstitution of the female pronucleus by the fusion of vesicles which doubtless arise from different chromosomes.

II. Fertilization.

The spermatheca opens into the oviduct at a point near the junction of the latter with the ovary. At the time when the eggs pass into the oviduct the spermatheca is distended with great numbers of spermatozoa a few of which are found in the narrow duct connecting spermatheca and oviduct. The spermatozoön meets the egg as it passes along the oviduct past the opening of the spermathecal duct.

Apparently the passage of the eggs through from the ovary to the uterus is periodical for among the large numbers of proglottids sectioned which contained cleavage-stages in the uterus and fully grown unfertilized eggs in the ovary only a very few show eggs in the oviduct. Among these, however, it has been possible to find a few cases showing the entrance of the sperm.

Fig. 1 (Pl. II.) shows the clearest case observed of this stage. It will be recalled[1] that the fully developed spermatozoön is greatly elongated and filiform without any trace of a visibly differentiated head-region, except that its diameter is slightly greater anteriorly than posteriorly.

In all cases where the spermatozoön was found on the egg it was in contact with the surface over more or less of its length, as if adhering to it. Frequently it was wound several times about the egg, the remaining portion hanging free in the oviduct or extending into the spermathecal duct.

In Fig. 1 the course of the spermatozoön body over the surface of the egg is indicated but only a fraction of the length of the spermatozoön is shown. The figure scarcely exaggerates the clearness of the section itself. That portion of the sperm which

[1] Child, Biol. Bull., XII, 4, 1907.

has entered the cytoplasm of the egg forms a deeply staining rounded mass in strong contrast to the remaining portions. Surrounding it is an area of cytoplasm staining less deeply than other portions of the egg — indicated in the figure by the dotted line. The appearance of the head-like structure is all the more remarkable since no trace of anything of the kind is visible before entrance. Evidently the anterior end of the spermatozoön loses its greatly elongated form after it enters the egg. It has been impossible to determine how much of the spermatozoön is involved in this change and also whether the remaining portions, if any, fuse with the cytoplasm or are cast off.

It is impossible to distinguish with certainty the male pronucleus from small yoke-spherules after the "tail" of the spermatozoön disappears. The earliest stages observed with anything like certainty are shown in Figs. 2, 3 and 7 (Pl. II.). By the time the egg reaches the stage of the second polar spindle, however, the male pronucleus can usually be found in some part. In the eggs shown in Figs. 9 and 10 (Pl. III.) it was present in other sections.

By the time maturation is completed the male pronucleus has attained large size and shows a faintly staining reticulum with a nucleolus of large size. Figs. 11–14 (Pl. III.) show the two pronuclei at various stages of approximation to each other.

It has not been possible to obtain the slightest evidence in support of the view that the cleavage centrosomes arise from the spermatozoön. In the early stages of the male pronucleus (Figs. 2, 3 and 7, Pl. II.) no trace of spheres or centrosomes has been observed in connection with it. In a number of cases the two centrosomes have been observed lying near the pronuclei before cleavage (Figs. 12, 13, 14, Pl. III.) but in no case was there the slightest indication that they were more closely associated with one nucleus than with the other.

There can be little doubt from these observations that the peculiar spermatozoa of *Moniezia* actually fertilize the eggs and therefore that they contain nuclear substance in some form or condition, or at least substance capable of giving rise under proper conditions to a nucleus. If future investigation shall establish what seems at least possible from my own observations, viz., that

functional spermatozoa arise from the "spermatid" nuclei, which themselves arise by fragmentation of the spermatocyte-nuclei,[1] we shall be forced to the conclusion that fertilization is possible without the typical process of maturation.

III. Cleavage.

The relations of the two pronuclei at the time of the first cleavage varies considerably in different eggs. In some cases the chromosomes form (Fig. 12, Pl. III.) and the membrane disappears while the pronuclei are still more or less widely separated. In such cases the chromosomes appear in two distinct groups on the two sides of the spindle (Figs. 15 and 16, Pl. IV.). In other cases the pronuclei approach more closely (Fig. 13, Pl. III.) or even undergo more or less complete fusion (Fig. 14, Pl. III.) before the disappearance of the membrane. In such cases the chromosomes may or may not appear in two groups according to the completeness of the fusion. In one case the chromosomes of the first cleavage were observed in two distinct groups within a single nuclear membrane (Fig. 17, Pl. IV.) and in many cases no trace of the paternal and maternal groups was visible (Figs. 18, 19, 20, Pl. IV.).

The position of the first cleavage-spindle varies greatly as is evident from Figs. 15, 16, 18, 19 and 20 (Pl. IV.). Since nuclear division proceeds much more rapidly than cytoplasmic division during early cleavage, and since the polar bodies soon degenerate or are absorbed, it has not been possible to determine whether the plane of the first cleavage is uniform in position, *i. e.*, whether the first cleavage-spindle finally attains a definite typical orientation in the egg. From my observations on cleavage stages I am somewhat inclined to doubt that this is the case, although my data are not conclusive.

There can be no doubt that the first cleavage is usually mitotic but occasionally conditions are found which might readily be regarded as cases of amitosis. Frequently eggs containing only a large single nucleus which is apparently giving rise amitotically to a smaller nucleus are found. None of these cases are figured since it is by no means certain that they are normal phenomena.

[1] Child, C. M., Biol. Bull., XII., 4, 1907.

In many proglottids a certain proportion of the cleaving eggs undergoes degeneration sooner or later, and such eggs usually show irregular nuclear fragmentations. It is therefore possible that the amitotic first cleavage may be an indication of degeneration. Possibly such eggs are not fertilized and are therefore incapable of normal development.

But although the first cleavage is usually or always mitotic, there can be no doubt that amitotic division appears very early in the course of cleavage.

In most cases a number of nuclear divisions occur before cell-boundaries become visible in the egg (Fig. 21, Pl. V.). Cases of mitosis are rarely seen after the first cleavage but amitosis is of frequent occurrence (Figs. 21–26, Pl. V.). It was impossible to determine whether the cleavage exhibited any regularity, for no basis for orientation was discovered. As cleavage proceeds, the egg is gradually divided into blastomeres containing yolk and blastomeres without yolk. In earlier stages the yolk-bearing blastomeres often contain two or more nuclei (Figs. 23, 26, Pl. V.), but in later stages after cytoplasmic cleavage is more advanced they usually contain one relatively large nucleus (Figs. 28, 29, 30, Pl. VI.). In other words as these yolk-bearing blastomeres are gradually reduced in size by successive cleavages the cytoplasmic cleavages keep pace more nearly with the nuclear divisions.

In the yolkless portions of the egg, however, nuclear division continues to be far in advance of cytoplasmic division as far as the cleavage has been followed (Figs. 27, 29, 30, 31, Pl. VI.; 32, Pl. VII.): the consequence is that each blastomere contains several or many nuclei of relatively small size. Evidently the nuclei in these yolkless portions of the egg are dividing much more rapidly than those in the yolk-bearing portions and, as is evident from the figures of Plates V. and VI., amitosis is the typical method of division.

Rarely a case of mitosis is observed: in all the hundreds of eggs in cleavage stages which have been examined, not more than a dozen cases of mitosis have been seen in stages later than the first cleavage. When mitosis occurs it apparently always involves one of the larger nuclei. Mitotic divisions of the small

nuclei in stages like those shown in Plate VI. have never been observed. The smaller nuclei are, without doubt, dividing more rapidly than the larger, and we are probably justified in concluding that amitosis occurs in those regions of the egg where division is most rapid, while mitosis is found, when it occurs at all, among the nuclei which are dividing more slowly.

In Figs. 28 and 30 (Pl. VI.) two cases of mitosis are figured. In these two cases, in fact in every case of mitosis observed during later cleavage, the spindle lies within a blastomere which is bounded on all sides by a distinct membrane. This is particularly well shown in Fig. 30 where the blastomere undergoing mitosis forms a spherical mass with distinct membrane in the midst of the egg-syncytium. This fact, like others mentioned in preceding papers,[1] seems to indicate that conditions in regions where mitosis occurs are widely different from those in which nuclear division is amitotic. Evidently some physical or chemical condition is present in the region about the mitotic spindle in Fig. 30 which determines the formation of a cell-membrane about a certain mass of the cytoplasm. It is not impossible that the membrane may be a coagulation-product resulting from difference in electrical condition of the colloids in the two regions. Suggestions of this nature have been made by various authors, both as regards nuclear membranes and cell-membranes of this character.

As is evident from the figures, the embryos are quite irregular in shape. As cleavage proceeds, however, elongation commonly occurs, but whether this elongation is a mechanical deformation resulting from pressure of the uterine walls as the egg is forced through narrow openings, or whether it is a typical feature of morphogenesis is not certain. Such cases as Fig. 32 (Pl. VII.) in which one end of the embryo is drawn out into a sharp point seem to indicate mechanical deformation.

Even in these later stages of cleavage it has not been possible thus far to discover any certain basis for orientation of the embryo. In many cases one large yolk-bearing blastomere is found at each end of the elongated embryo (Fig. 27, Pl. VI.). In other cases only one such blastomere appears (Figs. 30, 31, Pl. VI.). My

[1] Child, Biol. Bull., XII., 2, 3, 4, 1907.

observations certainly indicate, though they are not sufficient to prove positively, that cleavage is indeterminate and exceedingly irregular.

My material thus far has not included stages of development later than those shown in Plate VI. I hope in future, however, to obtain later stages and to continue the study of the rôle played by amitosis and mitosis in the later development.

In Figs. 33–40 (Pl. VII.) several single blastomeres and nuclei from various stages of cleavage are figured as showing particularly clear and convincing cases of amitosis.

IV. General Observations.

In this section a few observations of general biological interest though not connected with the chief purpose of the paper are briefly given. As was pointed out in the first paper of this series[1] the eggs in those portions of the ovary nearest the opening of the oviduct begin and complete their growth earlier than the others. Proceeding from this region toward the tips of the ovarian follicles, we find that oögenesis is slightly later at each successive level. Thus the eggs nearest the oviduct are the earliest, while those at the tips of the follicles are the latest to attain full growth.

There can be no doubt that the passage of ova from the ovary to the uterus is periodical and not of continual occurrence, for in most proglottids in which embryos are present in the uterus and immature eggs in the ovary, no eggs are found in the oviduct. In such proglottids nothing later than late maturation stages or cleavage stages is found in the uterus. Occasionally, however, a proglottid is found with eggs in the oviduct, and such cases show the entrance of the spermatozoa in those eggs in the oviduct and usually earlier maturation stages in the eggs in the lateral region of the uterus.

The egg usually reaches the lateral portions of the uterus before the appearance of the first maturation spindle. In the uterus, maturation stages and early cleavage stages become more or less mingled, although the maturation stages are usually more abundant in the lateral portions of the uterus and the cleavage stages in the middle portions.

[1] Child, C. M., Biol. Bull., XII., 2, 1907.

After passage of the eggs from ovary to uterus has occurred several times the different stages are mingled together in great confusion in the uterus for the uterus is now larger than in earlier stages and the eggs move more freely through it with the contractions of the body. In these proglottids particular stages are not confined to particular regions of the uterus: early maturation and later cleavage stages may occur side by side but even here the earlier stages are more abundant in the lateral and the later in the middle regions of the uterus.

That portion of the oviduct which lies between the entrance of the spermathecal duct and the uterus is highly convoluted and possesses thick walls, but no portion of it functions as a shell-gland. The egg passes into the uterus surrounded only by the vitelline membrane and this often disappears during cleavage.

Moreover, although a well developed vitellarium is present no yolk-cells pass into the uterus with the egg. To all appearances, the vitellarium undergoes degeneration *in situ*, though it is possible that yolk-cells may pass into the uterus in stages later than those which I have observed, or it may be that the reserve supplies of nutriment in the yolk-cells are altered by enzymes and so pass into the uterus in fluid form where they undergo resorption by the embryos.

It is very evident that the transference of the embryos of *Moniezia* to the intermediate host must occur either within the proglottid or in fluid since the absence of a shell or capsule of any kind would exclude the possibility of exposure to the atmosphere.

V. Conclusion.

Maturation and fertilization occur in *Moniezia* in a manner not essentially different from that observed in other forms. Evidently the earlier amitotic history of the germ-cells does not interfere in any way with their properties as germ-cells.

Moreover, although the embryonic development begins with mitosis, at least in most cases, if not in all, this soon gives place in large measure to amitosis, mitosis occurring only occasionally in the larger nuclei in the more slowly dividing regions. Here then, as in the development of the germ-cells, amitosis is appar-

ently connected with more rapid, and mitosis with less rapid division.

The following paper will include a brief account of the rôle of amitosis in the organogeny of the proglottid, together with a general discussion and attempt at interpretation of the data presented regarding amitosis and mitosis in *Moniezia*.

April, 1907.

EXPLANATION OF PLATE II.

FIG. 1. The entrance of the spermatozoön.

FIGS. 2 and 3. Preparation for first maturation spindle; male pronucleus near surface of egg.

FIGS. 4, 5 and 6. First maturation spindle.

FIG. 7. Resting stage of oöcyte-nucleus before second maturation division.

FIG. 8. Early stage of second maturation spindle.

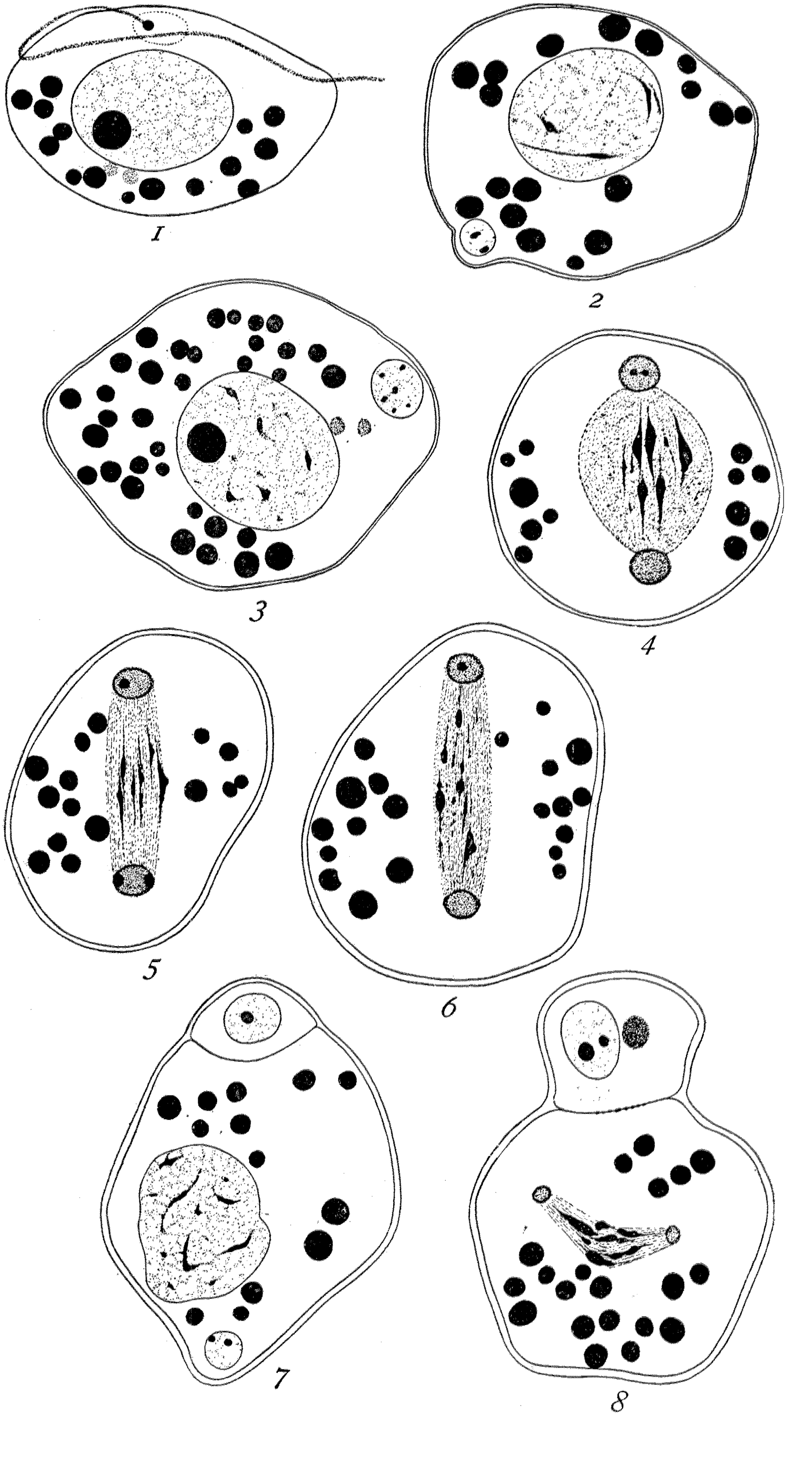
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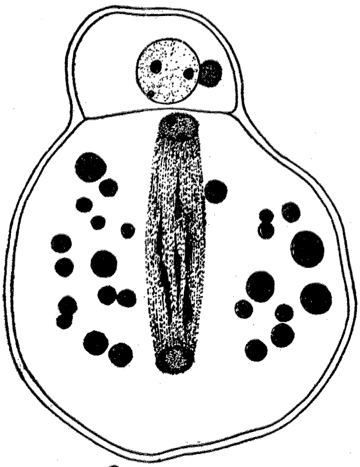
PLATE III.

FIG. 9. Second maturation spindle.

FIG. 10. Second maturation spindle; anaphase, a stage very rarely observed.

FIG. 11. Reconstitution of female pronucleus; male pronucleus on right.

FIGS. 12, 13 and 14. Male and female pronucleus before first cleavage.



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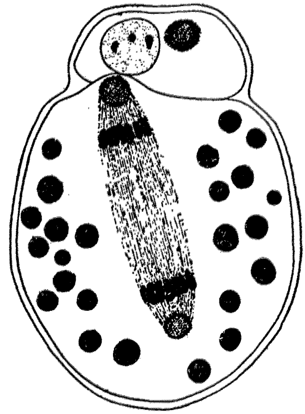

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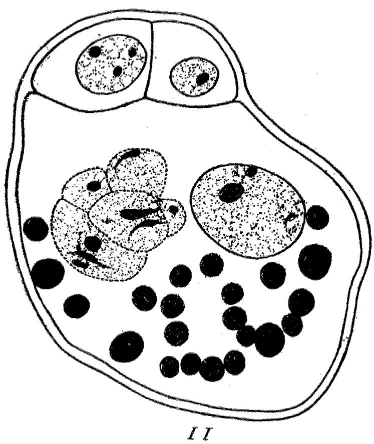

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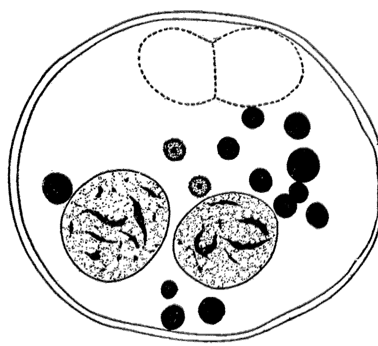

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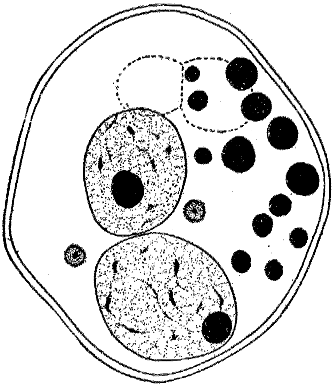

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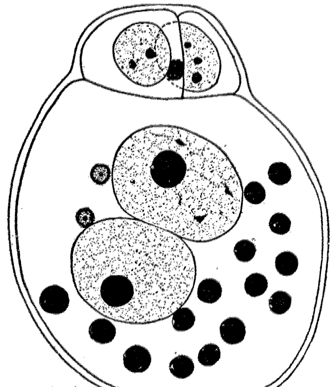

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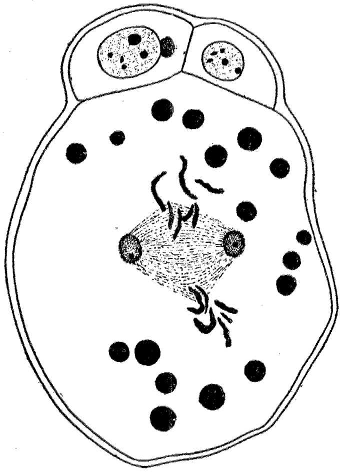
PLATE IV.

FIGS. 15, 16 and 17. Early stages of first cleavage, showing distinct paternal and maternal groups of chromosomes, in Fig. 17 both within a single nucleus.

FIGS. 18, 19 and 20. First cleavage, with chromosomes in a single group.

The figures show wide variation in the orientation of the first cleavage spindle.



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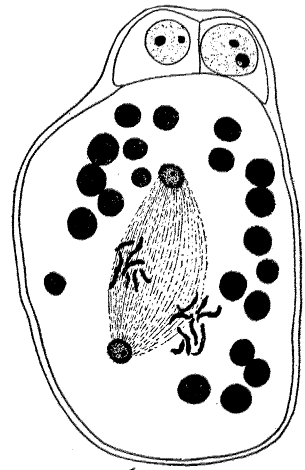

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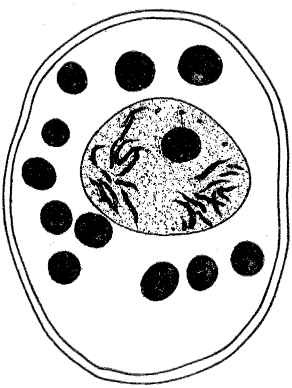

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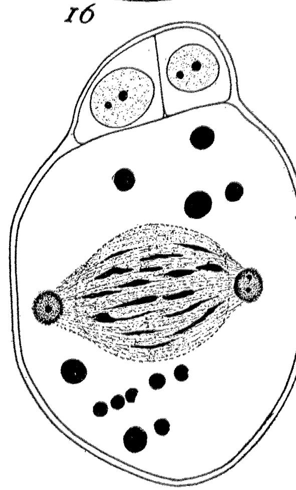

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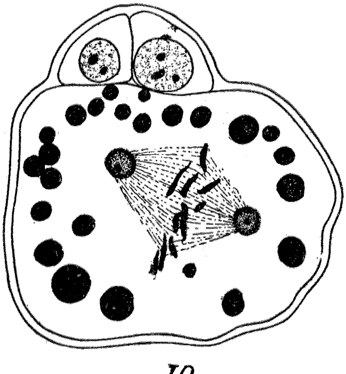

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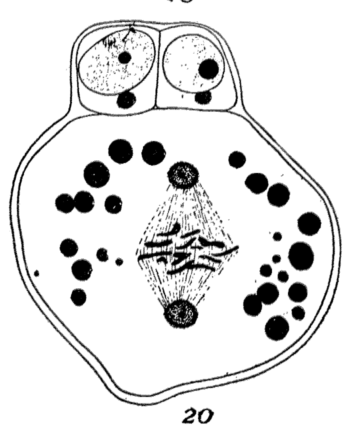

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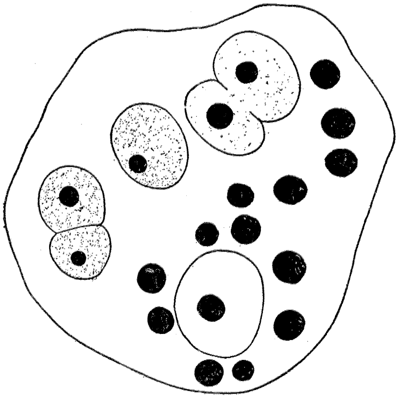
PLATE V.

FIGS. 21–26. Early cleavage-stages.



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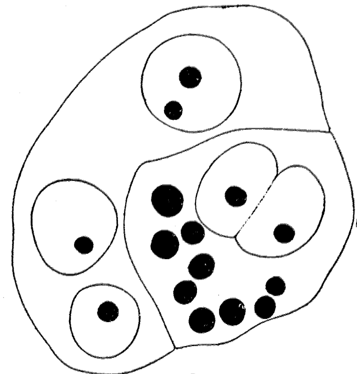

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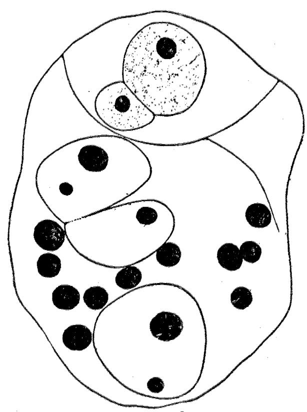

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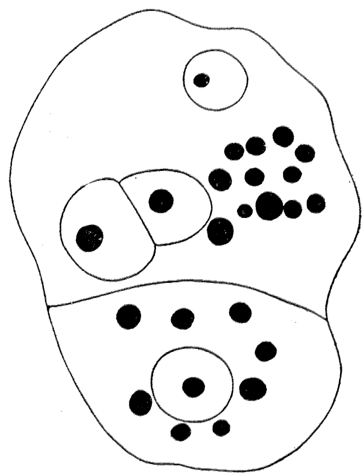

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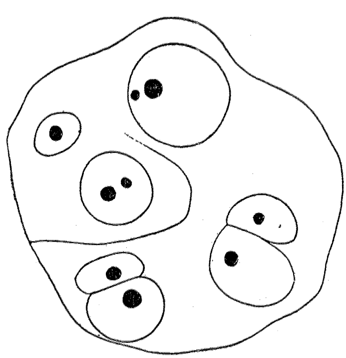

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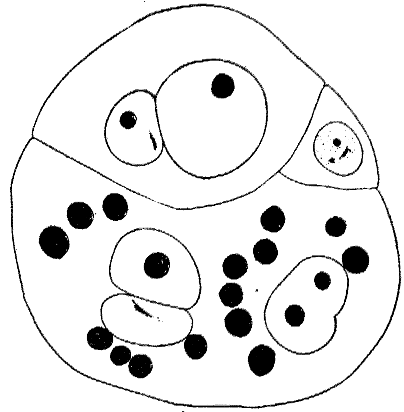

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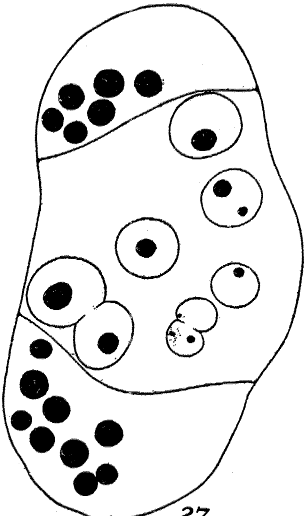
PLATE VI.

FIGS. 27–31. Later cleavage-stages.



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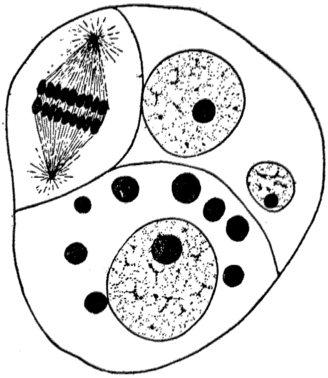

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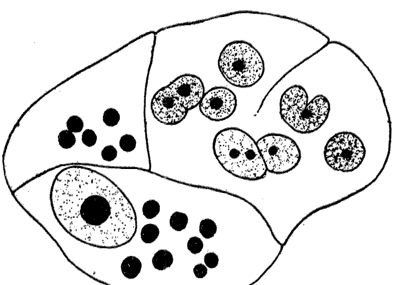

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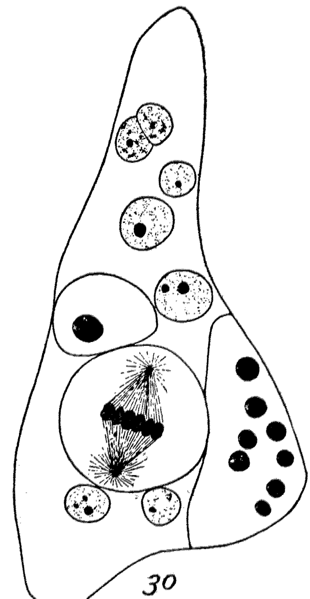

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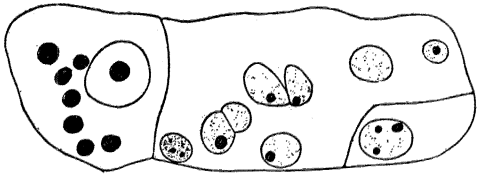

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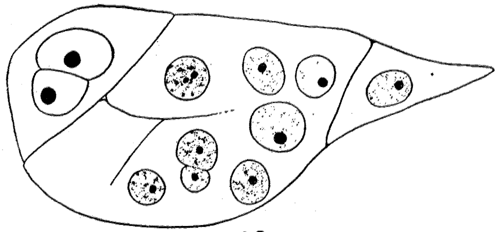
PLATE VII.

FIGS. 32–40. Cleavage-stages; all except Fig. 32 show single blastomeres or dividing nuclei.



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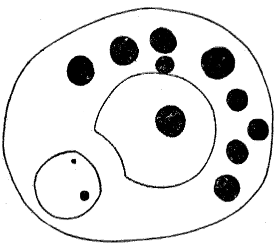

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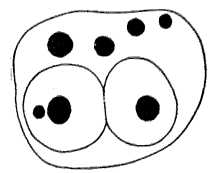

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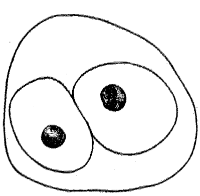

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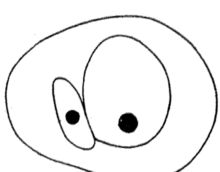

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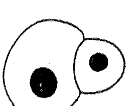

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